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Various calibration procedures result in optimal standardization of routinely used 25(OH)D ID-LC-MS/MS methods

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Abstract

Background—The variety of LC-MS/MS methods measuring total 25(OH)D used today is vast and the comparability among these methods is still not well assessed.

Methods—Here, we performed a comparison in samples of healthy donors between the currently routinely used 25(OH)D LC-MS/MS methods in the Netherlands and the Ghent University reference measurement procedure to address this issue (n = 40). Additionally, an interlaboratory comparison in patient serum samples assessed agreement between the Dutch diagnostic methods (n = 37).

Results—The overall correlation of the routine methods for 25(OH)D_3 with the reference measurement procedures and with the mean of all diagnostic methods was excellent ($r > 0.993$ and $r > 0.989$, respectively). Three out of five methods aligned perfectly with both the reference measurement procedure and the median of all methods. One of the routine methods showed a small positive bias, while another showed a small negative bias consistently in both comparisons.

Conclusion—The biases most probably originated from differences in calibration procedure and may be obviated by reassessing calibration of stock standards and/or calibrator matrices. In conclusion, five diagnostic centers have performed a comparison with the 25(OH)D Ghent University reference measurement procedure in healthy donor serum samples and a comparison among themselves in patient serum samples. Both analyses showed a high correlation and specificity of the routine LC-MS/MS methods, yet did reveal some small standardization issues

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that could not be traced back to the technical details of the different methods. Hence, this study indicates various calibration procedures can result in perfect alignment.

Keywords

Mass spectrometry; Method comparison; Standardization; Vitamin D

1. Introduction

Assessment of Vitamin D status in patients relies on accurate measurement of 25-hydroxyvitamin D (25(OH)D) concentration in serum or plasma, which can be achieved through appropriate standardization [2]. In a joined effort to implement standardized measurements for 25(OH)D, the National Institute of Health (NIH), the US Center for Disease Control and Prevention (CDC), the US National Institute of Standards and Technology (NIST) and the Belgian Laboratory for Analytical Chemistry in Ghent (UGhent), in collaboration with other researchers and organizations, established the Vitamin D Standardization Program (VDSP) [9]. The goal of this collaboration is to make 25(OH)D measurements traceable to the highest order reference, the NIST Standard Reference Material 2972a, by using recognized reference measurement procedures (RMP) operated at NIST, CDC, and UGhent and high quality serum based reference materials with values assigned by these RMPs [8, 10,11]. Although several immunoassays and liquid chromatography-tandem mass spectrometry (LC-MS/MS) assays have been standardized by CDC's Vitamin Standardization Certification Program [1], information about the accuracy of routinely used liquid chromatography-tandem mass spectrometry (LC-MS/MS) 25(OH)D methods is very limited. Since LC-MS/MS methods are known to generate more specific and accurate measurements than immunoassay-based methods, many laboratories have implemented this technique for patient assessment [4–6]. While in theory all LC-MS/MS based methods should deliver similar results, the actual procedures for preparing samples, standards or operating the instruments can be vastly different which leads to differences in measurement accuracy and performance. To address this matter, we performed a method comparison study between the currently used routine 25(OH)D LC-MS/MS methods in the Netherlands and the UGhent RPM using healthy donor serum samples and an inter-laboratory method comparison to assess agreement between the different laboratories using routine patient serum samples.

2. Materials and methods

2.1. Samples

Forty single healthy donor serum samples from the CDC Vitamin D Standardization Certification Program (VDSCP) (so called 'phase 1 samples'), which had been assigned a reference value by the UGhent RMP were used. These sera were obtained and processed according to CLSI protocol C37 [13] and covered a range of 23 to 198 nmol/L for $25(OH)D_3$, < 1 to 14 nmol/L for $25(OH)D_2$, and 2 to 43 nmol/L for epi-$25(OH)D_3$. In addition, 37 single patient donor serum samples were obtained by drawing an extra tube of blood from patients who already underwent a venipuncture for diagnostic purposes in our outpatient clinic (VU University Medical Center, Amsterdam). These patient sera had

concentrations ranging from <1 to 134 nmol/L for 25(OH)D_3, < 2 to 27 nmol/L for 25(OH)D_2 and < 1 to 7 nmol/L for epi25(OH)D_3 as determined by [12]. All samples were anonymized immediately after withdrawal and processed like regular patient samples. After centrifugation, serum was separated, aliquotted and frozen at −20 °C until analyses. Samples were distributed frozen on dry ice. Studies were approved by the local medical ethical committees.

2.2. Analytical methods

Five laboratories (the Radboud University Medical Center Nijmegen (Method A) (in duplicate), the University Medical Center Groningen (Method B) (in duplicate), the Canisius Wilhelmina Hospital in Nijmegen (Method C) (in singlicate), Medlon in Enschede (Method D) (in singlicate) and the VU University Medical Center in Amsterdam (Method E) (in duplicate) measured total 25(OH)D_3 concentrations with their respective routine LC-MS/MS methods. Duplicate or singlicate measurements were based on the way routine patient samples are measured in each laboratory. Methods B, C and E measured 25(OH)D_3 and 25(OH)D_2 while Method A and D only measured 25(OH)D_3. Technical details of the measurement and calibration procedures are given in Tables 1 and 2, respectively. The characteristics of the UGhent RMP have been described elsewhere [10,11].

Ideally, the comparisons would be based on total 25(OH)D, which is defined as the sum of 25(OH)D3 and 25(OH)D2. However, here we chose to compare the sum of 25(OH)D3 and epi-25(OH)D3 for the RMP and 25(OH)D3 for the routine LC-MS/MS 25(OH)D methods. We thus excluded 25(OH)D2 as it is rarely seen in patient samples in the Netherlands and two of the five routine 25(OH)D LC-MS/MS methods therefore do not include it in their routine measurements. Moreover, we included epi-25(OH)D3 for the RMP, because all Dutch routine LC-MS/MS 25(OH)D methods co-measure it with 25(OH)D3. By doing so we assured the optimal assessment of the routine Dutch LC-MS/MS 25(OH)D methods in clinical decision making.

2.3. Statistical analysis

25(OH)D_3 concentrations, as determined by the five Dutch LC-MS/MS methods, were compared with the sum of 25(OH)D_3 and epi-25(OH)D_3 values obtained by the RMP for the healthy donor serum samples (n = 40). For the inter-laboratory comparison in patient serum samples (n = 37), the median of all 25(OH)D_3 measurements was compared to the individual 25(OH)D_3 results.

Passing and Bablok regression analyses, Bland Altman plots and Pearson’s correlation coefficients were used to assess agreement in the method comparisons (MedCalc Software Ltd.).

3. Results

Healthy donor serum sample comparison of the five routine LC-MS/MS methods measuring 25(OH)D_3 to the UGhent RMP yielded the equations and Pearson’s correlation coefficients as depicted in Table 2. Figs. 1 and S1 show the corresponding Passing and Bablok regression analyses and Bland-Altman plots, respectively.

Regression analysis showed significantly deviating slopes for methods B (9%) and D (−10%). Mean biases ranged from −9.59% to 7.20%. Sample specific biases, as expressed in the limits of agreement obtained from the bias plot analysis, ranged from ±10.63% to ±20.64%. All routine methods showed excellent correlations with values above 0.993. Passing and Bablok regression analysis of the median values of all routine LC-MS/MS methods in the healthy donor serum samples with the RMP resulted in a slope of 1.00 and a small mean bias of 1.54%. Sample specific variation was also modest with ±7.60%. An optimal correlation of 1.000 was observed.

Results of the comparison of $25(OH)D_3$ concentrations in patient samples of the five LC-MS/MS methods with the median of the measurements is shown in Fig. 2, Fig. S2 and Table 3 and yielded analogous results. Here, only method D showed a significant proportional bias of −10%. Mean biases were observed ranging from −13.85% to 5.01%, while sample specific bias ranged from ±9.59% to ±45.36%. Again high correlations were observed ($r \geq 0.989$).

4. Discussion

In this study we assessed the agreement of five routine 25(OH)D LC-MS/MS methods for their measurement of $25(OH)D_3$. To this end we compared the results of these methods to the UGhent RMP using single healthy donor serum samples and to the median of all methods in patient sera from routine analysis.

To correctly evaluate agreement between the routine 25(OH)D LC-MS/MS methods with the RMP, routine method $25(OH)D_3$ results were compared with the sum of $25(OH)D_3$ and epi-$25(OH)D_3$ for the RMP. Though normally total 25(OH)D (sum of $25(OH)D_3$ and $25(OH)D_2$) would be used, we opted for this approach as $25(OH)D_2$ is rarely seen in Dutch patient samples and two of the five routine 25(OH)D LC-MS/MS methods therefore do not include it in their routine measurements. Furthermore, since the routine 25(OH)D methods co-measure epi-$25(OH)D_3$ with their $25(OH)D_3$, we also summed $25(OH)D_3$ and epi-$25(OH)D_3$ for the RMP. In the first comparison 9 samples contained measurable $25(OH)D_2$ levels, which comprised on average 6% of the total 25(OH)D. All but two samples contained measurable epi-$(OH)D_3$ levels, comprising on average 6% of the total 25-(OH)D. The routine LC-MS/MS 25(OH)D methods not separating the $25(OH)D_3$ epimer will overestimate the sum of the $25(OH)D_3$ and epi-$25(OH)D_3$ concentrations as the two show slightly different ionization intensities [12]. However, if the average epi-$25(OH)D_3$ concentration comprises 6% of the $25(OH)D_3$ levels and the difference in ionization intensity is 30–40%, this would mean an estimated positive bias of approximately 2% for the routine 25(OH)D methods, which can be considered clinically irrelevant. It is thought that only when measuring in infants, the epi-$25(OH)D_3$ concentrations comprise high enough percentages of $25(OH)D_3$ to be of relevance in clinical decision making [7,14]. Though some reports suggests that the epi-25(OH)D3 concentration is not negligible in all adults [15,16]. All things considered, this study therefore focused on $25(OH)D_3$.

The 25(OH)D LC-MS/MS methods, correlated very well with the sum of $25(OH)D_3$ and epi-$25(OH)D_3$ for the RMP ($r \geq 0.993$) and with the median $25(OH)D_3$ results of the five

routine methods ($r \geq 0.989$). This is in accordance with earlier LC-MS/MS comparisons for $25(OH)D_3$ that have shown good correlations among themselves [4–6]. The comparison in patient donor serum was conducted to monitor any complications that may arise when measuring patient populations [3]. However, no such difficulties were observed, as evident from the agreement between the two sets of samples. Although all methods strongly correlated with the RMP, not all methods perfectly aligned with it. Both method B and D supposedly suffer from, albeit small, calibration issues as evident from the deviating slopes and reported mean biases in the Passing and Bablok regression analysis and the Bland-Altman plots, respectively. For these two labs, mean biases exceeded the performance criterion of ±5% mean bias as drafted by the CDC. Similar findings were reported following the interlaboratory comparison, where the median values of all five laboratories served as reference. The use of the median as reference was justified, as is showed by the perfect alignment (slope of 1.00) and correlation ($r = 1.000$) with the RMP in the first comparison. In the interlaboratory comparison, Methods B and D again showed positive and negative slope deviations, respectively. For method B, the deviating slope was no longer significant. As expected, the correlation, although still very high, decreased slightly with this second comparison ($r \geq 0.989$).

The observed differences may originate from the slightly different technical details as described in Table 1. Though, as all assays included the use of an internal standard, neither equipment nor technician handling should contribute to the discrepancy. Calibration or preparation of stock standards, however, may influence mean bias. The observed differences in sample specific biases, as represented by the limits of agreement, markedly differed between the routine methods. Variance in operation procedure and/or data processing may be causative for this. We performed a detailed analysis of the various calibration procedures performed by the five centers to assess if additional attention to avoid potential biases is required. Nonetheless we were unable to explain the observed biases from the technical details of any the routine methods. In fact, as Methods A, C and E show no uniformity in their technical details, yet show optimal alignment and excellent correlation in both comparisons, proper calibration is possible through multiple means, without one preferable over the other. Hence, other potential sources of bias need to be investigated.

In conclusion, five diagnostic centers measuring total 25(OH)D have performed a comparison for $25(OH)D_3$ with the UGhent RMP in healthy donor serum samples and a comparison among themselves in patient serum samples. Both analyses showed a high correlation and specificity of LC-MS/MS methods, yet did reveal some small standardization issues that could not be traced back to the technical details of the different methods. Hence, this study indicates various calibration procedures can result in perfect alignment with the RMP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Aabbreviations

LC-MS/MS	liquid chromatography-tandem mass spectrometry
25(OH)D	25-hydroxyvitamin D
NIH	National Institute of Health
CDC	Centers for Disease Control and Prevention
NIST	US National Institute of Standards
UGhent	Belgian Laboratory of Analytical Chemistry in Ghent
VDSP	Vitamin D Standardization Program
RMP	reference measurement procedure
SKML	Dutch Foundation for Quality Assessment in Medical Laboratories

References

1. Cdc certified vitamin D procedures. from http://www.cdc.gov/labstandards/pdf/hs/CDC_Certified_Vitamin_D_Procedures.pdf
2. Cashman KD, Kiely M, Kinsella M, Durazo-Arvizu RA, Tian L, Zhang Y, et al. Evaluation of vitamin D standardization program protocols for standardizing serum 25-hydroxyvitamin D data: a case study of the program's potential for national nutrition and health surveys. Am J Clin Nutr. 2013; 97:1235–1242. [PubMed: 23615829]
3. Depreter B, Heijboer AC, Langlois MR. Accuracy of three automated 25-hydroxyvitamin D assays in hemodialysis patients. Clin Chim Acta. 2013; 415:255–260. [PubMed: 23159781]
4. Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. Clin Chem. 2012; 58:531–542. [PubMed: 22230812]
5. Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin d binding protein concentration. Clin Chem. 2012; 58:543–548. [PubMed: 22247500]
6. Janssen MJ, Wielders JP, Bekker CC, Boesten LS, Buijs MM, Heijboer AC, et al. Multicenter comparison study of current methods to measure 25-hydroxyvitamin D in serum. Steroids. 2012; 77:1366–1372. [PubMed: 22925701]
7. Keevil B. Does the presence of 3-epi-25ohd3 affect the routine measurement of vitamin D using liquid chromatography tandem mass spectrometry? Clin Chem Lab Med. 2012; 50:181–183.
8. Mineva EM, Schleicher RL, Chaudhary-Webb M, Maw KL, Botelho JC, Vesper HW, et al. A candidate reference measurement procedure for quantifying serum concentrations of 25-hydroxyvitamin D(3) and 25-hydroxyvitamin D(2) using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2015; 407:5615–5624. [PubMed: 25967149]
9. Sempos CT, Vesper HW, Phinney KW, Thienpont LM, Coates PM, Vitamin DSP. Vitamin D status as an international issue: national surveys and the problem of standardization. Scand J Clin Lab Investig Suppl. 2012; 243:32–40. [PubMed: 22536760]
10. Stepman HC, Vanderroost A, Van Uytfanghe K, Thienpont LM. Candidate reference measurement procedures for serum 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 by using isotope-dilution

liquid chromatography-tandem mass spectrometry. Clin Chem. 2011; 57:441–448. [PubMed: 21248072]

11. Tai SS, Bedner M, Phinney KW. Development of a candidate reference measurement procedure for the determination of 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum using isotope-dilution liquid chromatography-tandem mass spectrometry. Anal Chem. 2010; 82:1942–1948. [PubMed: 20136128]
12. van den Ouweland JM, Beijers AM, van Daal H. Overestimation of 25-hydroxyvitamin D3 by increased ionisation efficiency of 3-epi-25-hydroxyvitamin D3 in lc-ms/ms methods not separating both metabolites as determined by an lc-ms/ms method for separate quantification of 25-hydroxyvitamin D3, 3-epi-25-hydroxyvitamin D3 and 25-hydroxyvitamin D2 in human serum. J Chromatogr B Anal Technol Biomed Life Sci. 2014; 967:195–202.
13. Wayne P. Preparation and Validation of Commutable Frozen Human Serum Pools as Secondary Reference Materials for Cholesterol Measurement Procedures (Clsi Document c37-a). 1999
14. Wright MJ, Halsall DJ, Keevil BG. Removal of 3-epi-25-hydroxyvitamin D(3) interference by liquid chromatography-tandem mass spectrometry is not required for the measurement of 25-hydroxyvitamin D(3) in patients older than 2 years. Clin Chem. 2012; 58:1719–1720. [PubMed: 23071364]
15. Lensmeyer G, Poquette M, Wiebe D, Binkley N. The C-3 epimer of 25-hydroxyvitamin D(3) is present in adult serum. J Clin Endocrinol Metab. 2012; 97:163–168. [PubMed: 22013102]
16. Wiebe D, Binkley N. Case report: three patients with substantial serum levels of 3-epi-25(OH)D including one with 3-epi-25(OH)D2 while on high-dose ergocalciferol. J Clin Endocrinol Metab. 2014:1117–1121. [PubMed: 24476080]

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2016.08.016.

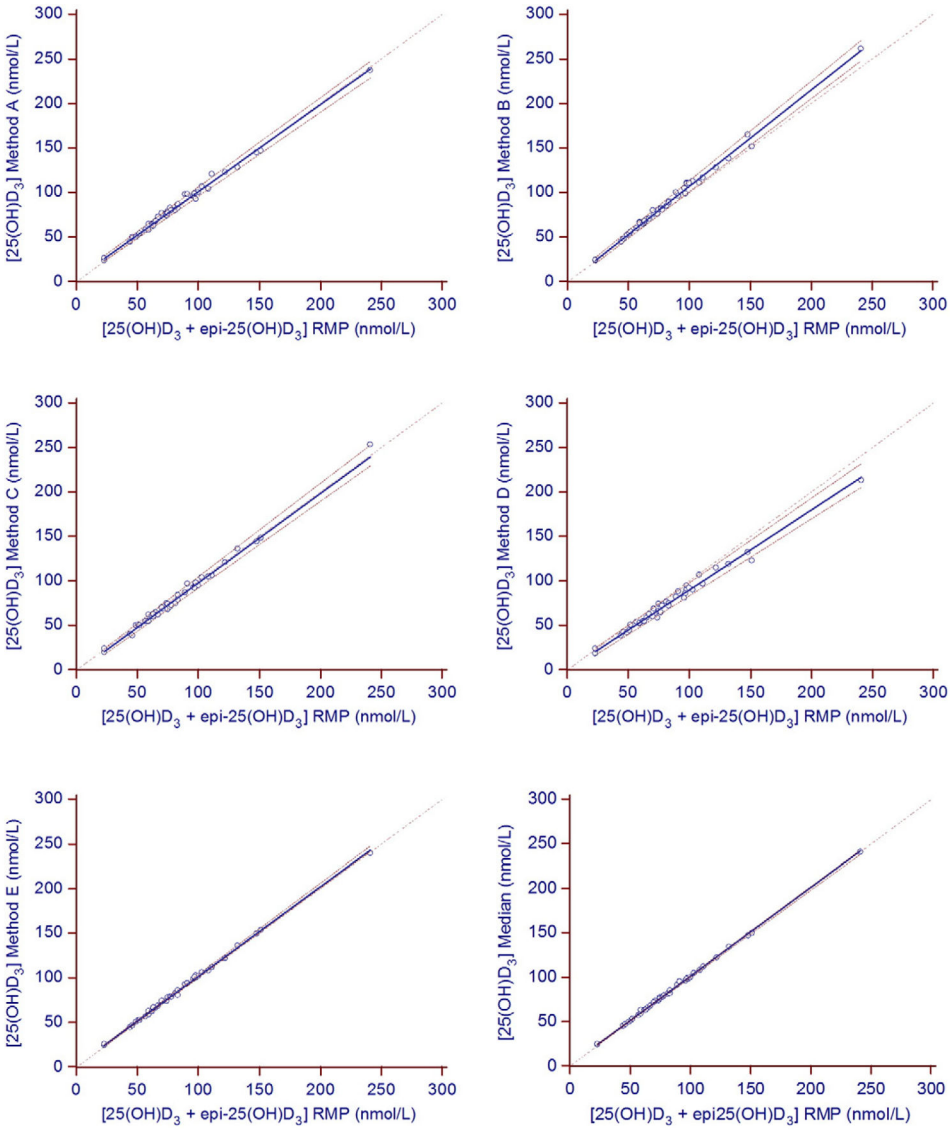

Fig. 1.

Passing and Bablok regression analyses of the five routine 25(OH)D LC-MS/MS methods measuring $25(OH)D_3$ in healthy donor serum samples and the median compared to the results obtained by the RMP (sum of $25(OH)D_3$ and epi-$25(OH)D_3$). Middle dotted line represents y = x, outer dotted lines represent 95% confidence interval. RMP, reference measurement procedure.

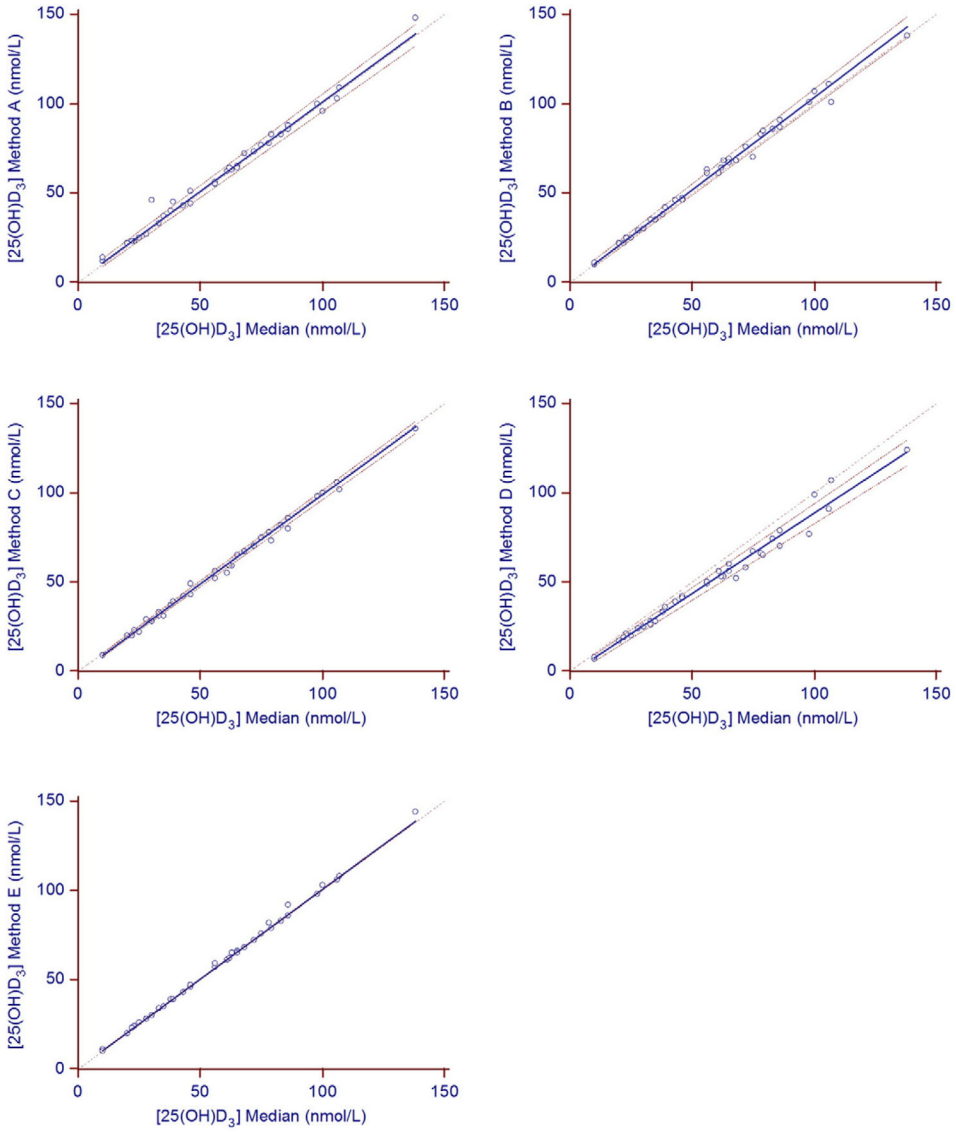

Fig. 2.

Passing and Bablok regression analyses of the five routine LC-MS/MS methods measuring $25(OH)D_3$ in patient serum samples compared to median of all methods. Middle dotted line represents y = x, outer dotted lines represent 95% confidence interval.

Table 1

Technical details of LC-MS/MS methods measuring total 25(OH)D.

	Instrument	Analytical column	Sample preparation	Internal standard		Measured quantifier transitions (*m/z*)				Calibrator matrix	Calibrator source	Calibration range (nmol/L)	
				25(OH)D_3	25(OH)D_2	25(OH)D_3	SIL* 25(OH)D_3	25(OH)D_2	SIL 25(OH)D_2			25(OH)D_3	25(OH)D_2
Method A	Agilent 1290 Infinity VL UPLC and Agilent 6490 Triple Quad LC/MS	Waters ACQUITY UPLC® BEH C18 1.7 µm 2.1 × 50 mm	Protein dissociation and precipitation using NaOH and acetonitrile/ methanol and subsequent SPE extraction (HLB 1 cm^3 10 mg extraction cartridges, waters)	$^2H_3$–25(OH)D_3	n.a.	401.4 → 159.2	404.2 → 162.1	n.a.	n.a.	Mobile phase	Gravimetric (25(OH)D_3 from Sigma) correction based on UV absorbance (at 18,200)	3.91–250	n.a.
Method B	Spark Holland Symbiosis system and Waters Quattro Premier XE MS/MS	Phenomenex, Synergi 4 µm Hydro-RP 80 Å, 2 × 100 mm	Protein disrupting buffer and online SPE using HySphere C_8 EC-SE, 10 µm, 2 × 10 mm cartridges	$^2H_6$–25(OH)D_3	$^2H_6$–25(OH)D_2	401.2 → 257.25	407.2 → 263.25	413.2 → 159.15	419.2 → 159.15	25(OH)D_3 depleted plasma	Calibrated using Standard Reference Material 2972 25-Hydroxyvitamin D_2 and D_3 Calibration solutions.	9.60–192	9.30–186
Method C	Waters Acquity UPLC and Waters Quattro Premier XE MS/MS	Waters ACQUITY UPLC® BEH C18 1.7 µm 2.1 × 50 mm	Protein precipitation using acetonitril/ methanol followed by SPE (Oasis HLB)	$^2H_6$–25(OH)D_3	$^2H_3$–25(OH)D_2	401.1 → 159.0	407.3 → 159.0	413.3 → 159.0	416.3 → 82.8	6% BSA in PBS	Calibrated using Standard Reference Material 2972 25-Hydroxyvitamin D_2 and D_3 Calibration solutions.	2.5–500	2.0–500
Method D	Shimadzu LC 20 AD and QTRAP 3200 (AB SCIEX)	Agilent, Zorbax SB-C8, 3.5 µm, 3 × 100 mm	Protein precipitation using acetonitril followed by heated evaporation and reconstitution in 70% MeOH	$^2H_3$–25(OH)D_3	n.a.	383.4 → 257.4	389.4 → 263.4	n.a.	n.a.	MeOH	Calibrated using H-083 CERILLIANT 25-Hydroxyvitamin D3 solution (Sigma)	25–250	n.a.
Method E	Waters Acquity UPLC and Waters Quattro Premier XE MS/MS	Phenomenex, Kinetex C18, 2.6 µm, 2.1 × 75 mm	Protein precipitation using acetonitril and liquid-liquid extraction using hexane	$^2C_5$–25(OH)D_3	$^2H_6$–25(OH)D_2	401.1 → 383.3	406.1 → 388.3	413.2 → 395.2	419.2 → 401.2	6% BSA in PBS	Gravimetric (25(OH)D_3 and 25(OH)D_2 from Toronto Research Chemicals)	4.27–346	1.31–106

*SIL, Stable-isotope-labelled.

Table 2

Characteristics of the method comparison in donor serum samples (N = 40) of the five diagnostic centers and their median with the UGhent RMP.

	Passing and Bablok regression		Blant Altman		Pearson's correlation
	Slope (95% CI)	**Intercept (95% CI)**	**Mean bias (95% CI)**	**Limits of agreement (%)**	**r (95% CI)**
Method A	0.97 (0.94–1.00)	3.88 (2.00–6.25)	3.27 (1.77–4.77)	−5.94–12.47	0.996 (0.993–0.998)
Method B	1.09 (1.05–1.13)	−1.56 (−4.62–1.20)	7.20 (6.10–8.29)	0.49–13.90	0.997 (0.993–0.998)
Method C	1.00 (0.95–1.04)	−1.50 (−4.73–1.83)	−2.61 (−4.10–1.11)	−11.77–6.56	0.996 (0.992–0.998)
Method D	0.90 (0.86–0.95)	0.39 (−2.97–4.07)	−9.59 (−11.30–7.83)	−0.73–19.91	0.993 (0.986–0.996)
Method E	1.00 (1.00–1.02)	2.00 (0.48–2.00)	3.03 (2.16–3.90)	−2.29–8.34	0.999 (0.999–1.000)
Median	1.00 (0.98–1.00)	1.00 (1.00–1.98)	1.54 (0.92–2.16)	−2.26–5.34	1.000 (0.999–1.000)

Table 3

Characteristics of the method comparison in patient serum samples (N = 37) of the five diagnostic centers with the median.

	Passing and Bablok regression		Blant Altman		Pearson's correlation
	Slope (95% CI)	**Intercept (95% CI)**	**Mean bias (95% CI)**	**Limits of agreement**	**r (95% CI)**
Method A	1.00 (0.96–1.03)	1.00 (−0.60–2.98)	5.01 (1.10–8.93)	−17.67–27.69	0.993 (0.987–0.997)
Method B	1.04 (1.00–1.06)	−0.01 (−1.06–2.00)	3.77 (2.31–5.23)	−4.70–12.24	0.996 (0.992–0.998)
Method C	1.00 (0.98–1.00)	−1.00 (−1.46–0.27)	−3.36 (−4.87–1.85)	−12.10–5.38	0.998 (0.995–0.999)
Method D	0.90 (0.86–0.94)	−1.20 (−3.03–0.14)	−13.85 (−15.91–11.80)	−25.76–1.95	0.989 (0.979–0.995)
Method E	1.00 (1.00–1.00)	0.50 (0.50–0.50)	1.87 (1.04–2.70)	−2.92–6.67	0.999 (0.998–1.00)